

Taxonomic Studies on *Asplenium* sect. *Thamnopteris* (Aspleniaceae) I: Cytological Observations

SHI-YONG DONG

South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China;
dongshiyong@scib.ac.cn

ABSTRACT.—The section *Thamnopteris* is morphologically distinct among the large fern genus *Asplenium*, but the species recognition within this section is very difficult. To understand the species concept of this section from a cytological view, I examined chromosome numbers of fifteen samples representing eight taxa (species and intraspecific taxa). As result, seven taxa were determined to be sexual tetraploids with chromosome number $2n = 144$ and *Asplenium humbertii* is a sexual hexaploid with $2n = 216$. Along with the chromosome numbers reported, notes on nomenclature, diagnostic characters, and geographical distribution for the eight taxa are given. Cytological data available so far indicate that *Asplenium* sect. *Thamnopteris* is not a monophyletic group.

KEY WORDS.—Bird-nest fern, chromosome, polyploid, taxonomy

Asplenium L. is a natural genus with about 700 or more species in various regions all over the world (Kramer and Viane, 1990; Bellefroid *et al.*, 2010). Though widely accepted as monophyletic, *Asplenium*, with so many species, is difficult to subdivide because of a lack of suitable distinguishing characters at the intrageneric level. *Asplenium* sect. *Thamnopteris* C. Presl (1836), or treated as a separate genus *Neottopteris* (Smith, 1841; Wu, 1999), however, is one of the very few groups which can be clearly recognized. It is characterized by simple, entire fronds and intramarginal veins in morphology, is usually epiphytic on tree trunks or rocks in forests with bird-nest-like appearance, and is distributed in tropics and subtropics of the Old World.

Despite being readily recognized as a morphologically distinct group, the circumscription of species is confusing and the identification is very difficult within *Asplenium* sect. *Thamnopteris*. Since Linnaeus's *A. nidus* (1753), the first member in this group, 33 taxa have been published (Tardieu-Blot, 1933; Ching and Wang, 1964; Holttum, 1974; Miao, 1980; Jones, 1988; Wu, 1989; Murakami *et al.*, 1999). The establishment of these taxa was mostly based on a minor variation of one or two characters, especially the shape of fronds, which results in the obscure delimitation of species. Though Holttum (1974) presented a taxonomic revision on the section *Thamnopteris*, his treatment was based on herbarium specimens at his time and many problems in nomenclature and taxonomy still remained. After Holttum (1974), no taxonomic work was carried out on this group other than that of Seto (1979) and Murakami *et al.* (1999) who studied the morphology and the molecular systematics of *Thamnopteris* from Japan, respectively. Lacking a comprehensive comparison of characters, the taxonomic confusion of this section is partly reflected by the present situation that many specimens were named as

“*Asplenium nidus* L. s.l.” or “*A. phyllitidis* sensu Holttum” and more than 20 new species were proposed but not formally published based on materials in various herbaria.

Aiming to revise the taxonomy of this difficult group, the present author undertook examining type collections, studying materials in large herbaria of southeastern Asia and Europe, field surveying in China, Philippines and Indonesia, chromosome counting, and gametophyte observations in last six years. Cytological data are necessary and important to understand the concepts and relationships of species and to date only *A. antiquum* Makino, *A. australasicum*, *A. nidus*, and *A. phyllitidis* D. Don have been documented with chromosome numbers $n = 72$ and / or $2n = 144$ (Bir, 1960; Abraham *et al.*, 1962; Kawakami, 1970, 1997; Tsai and Shieh, 1983; Kato and Nakato, 1999; Yatabe *et al.*, 2001; Tindale and Roy, 2002). These cytological observations are reported as the first part of my serial studies on the taxonomy of *Asplenium* sect. *Thamnopteris*.

MATERIAL AND METHODS

Gross morphology and identification.—In order to correctly name the living materials involved in this research, gross morphology was studied based on herbarium specimens and wild and cultivated living plants. The present author examined specimens of *Asplenium* sect. *Thamnopteris* deposited in the following herbaria: BM, BO, GAUA, HITBC, IBK, IBSC, K, KUN, KYO, L, P, PE, PNH, PYU, and SING before the year 2009. The specimens of the section of *Thamnopteris* being examined in above herbaria amount to about 2500 collections. To understand the living state and variation of morphological characters, I made field observations in 17 trips to southern and southwestern China since 2005, one trip to Mindanao, Philippines in 2007, one trip to Java, Indonesia in 2009, and one trip to West Papua and Kalimantan, Indonesia in 2010. In each field trip, the state of following characters was determined or measured: distribution, shape, color, and size of scales; outline, size, and basal shape of fronds; prominent extent and transaction of frond midribs; distribution, length, and density of sori; color of stipes; spreading angle of fronds; and habitat. In addition, one or two living plants were introduced to my green house in South China Botanical Garden, Guangzhou, for morphological observation and chromosome counts.

Cytology.—Root tips of sporophytes or prothalli in gametophyte phase were used for chromosome counts. Living plants (sporophytes) or fresh spores were collected by me from natural populations in various localities or from Chinese gardens (Table 1) and cultivated in my green house in South China Botanical Garden. Fresh root tips or immature prothalli (nearly cordate in shape, before sex organs formed) raised from spores were pretreated for 3–6 hours with 0.002 mol/L 8-hydroxyquinoline at about 25°C, then fixed for 1 hour in Carnoy's fluid (1 volume of pure acetic acid and 3 volumes of 95% ethanol) at about 4°C. The tips were hydrolyzed for 10 minutes in 1 N HCL at 60°C, macerated for 10 min in 45% acetic acid, and then stained in 2% aceto-orcein

TABLE 1. Chromosome counts for 15 samples representing eight taxa in *Asplenium* sect. *Thamnopteris*.

Taxon	Cytological material	Chromosome count (ploidy level)	Collection locality and voucher	Figure
<i>A. antiquum</i>	Prothalli	n = 72 (4×)	South China Botanical Garden (in cultivation), China; <i>Dong 3360</i> (IBSC)	1
	Root tips	2n = 144 (4×)	Xiamen Botanical Garden (in cultivation), Fujian, China; <i>Dong 2894</i> (IBSC)	-
<i>A. antrophyoides</i>	Root tips	2n = 144 (4×)	Huanjiang, Guangxi, China; <i>Dong 1976</i> (IBSC)	2
<i>A. australasicum</i>	Root tips and prothalli	2n = 144, n = c 72 (4×)	South China Botanical Garden (in cultivation), Guangdong, China; <i>Dong 3400</i> (IBSC)	3
<i>A. cymbifolium</i> f. <i>lingganum</i>	Root tips and prothalli	2n = 144, n = c 72 (4×)	Marilog, Davao, Mindanao, Philippines; <i>Dong 2610</i> (IBSC)	4
<i>A. humbertii</i>	Root tips	2n = 216 (6×)	Longzhou, Guangxi, China; <i>Dong 2287B</i> (IBSC)	5
	Root tips	2n = c 216 (6×)	Mt Exianling, Hainan, China; <i>Dong 1234</i> (IBSC)	-
<i>A. nidus</i>	Root tips	2n = c 144 (4×)	Gongshan, Yunnan, China; <i>Dong 3402</i> (IBSC)	-
	Root tips	2n = c 144 (4×)	Mt Yinggeling, Hainan, China; <i>Dong 1667</i> (IBSC)	-
	Root tips	2n = 144 (4×)	Napo, Guangxi, China; <i>Dong 2939</i> (IBSC)	6
	Prothalli	n = c 72 (4×)	South China Botanical Garden, China; <i>Dong 3401</i> (IBSC)	-
<i>A. phyllitidis</i> subsp. <i>malesicum</i>	Root tips and prothalli	2n = 144, n = c 72 (4×)	Mt Yinggeling, Hainan, China; <i>Dong 1645</i> (IBSC)	7
	Root tips and prothalli	2n = 144, n = c 72 (4×)	Marilog, Davao, Mindanao, Philippines; <i>Dong 2606</i> (IBSC)	-
	Root tips	2n = 144 (4×)	South China Botanical Garden (in cultivation), China; <i>Dong 3360</i> (IBSC)	8
<i>A. simonsianum</i>	Root tips	2n = 144 (4×)	Mengla, Yunnan, China; <i>Dong 2768</i> (IBSC)	9

for 1-2 hours. Finally, the root tips or prothalli were squashed in 2% aceto-orcein. The chromosomes were counted and photographed using a light microscope (Olympus BX41).

Reproductive characteristics.—The number of spores in a sporangium indicates the possible reproduction mode for most leptosporangiate ferns, i.e., ferns with 64 spores per sporangium reproduce sexually while those with 32 spores per sporangium reproduce apogamously (Manton, 1950; Lovis, 1977;

Walker, 1979; Kato and Nakato, 1999). When checking the number of spores, a well-developed, intact sporangium was selected and moved to a drop of water on a microscope slide with a needle. Using the needle to break the sporangium, spores were freed from the sporangium, and then the number of spores was counted with a light microscope (Olympus BX41). For one given specimen, the spores of at least five sporangia were counted.

The data on morphology and distribution given in the following section is entirely based on herbarium specimens examined and recent collections gathered by the present author.

RESULTS AND DISCUSSION

As shown in Table 1, the somatic chromosome number is 216 for *Asplenium humbertii* and 144 for other species. As the basal number of chromosome (x) is 36 in *Asplenium*, *A. humbertii* is hexaploid and others are all tetraploid. In addition, my examination showed there are 64 spores in each sporangium and the spores can normally develop and produce new sporophytes for the all specimens examined cytologically in this study. Therefore, the eight species with chromosomes counted here are of sexual reproduction mode.

CHROMOSOME NUMBER, NOMENCLATURE, IDENTIFICATION, AND DISTRIBUTION

Asplenium antiquum Makino in J. Jap. Bot. 6: 32. 1929. *Neottopteris antiqua* (Makino) Masam. in Trans. Nat. Hist. Soc. Taiwan. 22: 215. 1932. TYPE.—JAPAN. Kagoshima: Yakusima, 30 Jul 1961, *H. Ito* 289 (neotype! designated here, SING).

Cytology.—The plants of this species cultivated in two botanical gardens of China were examined and determined to be tetraploid with $n = 72$ (Fig. 1) and $2n = 144$. The origin of the plants examined is unknown. Previously, a population of this species from Tokunoshima Isl., Japan, was reported with the same chromosome number ($2n = 144$) (Kawakami, 1970).

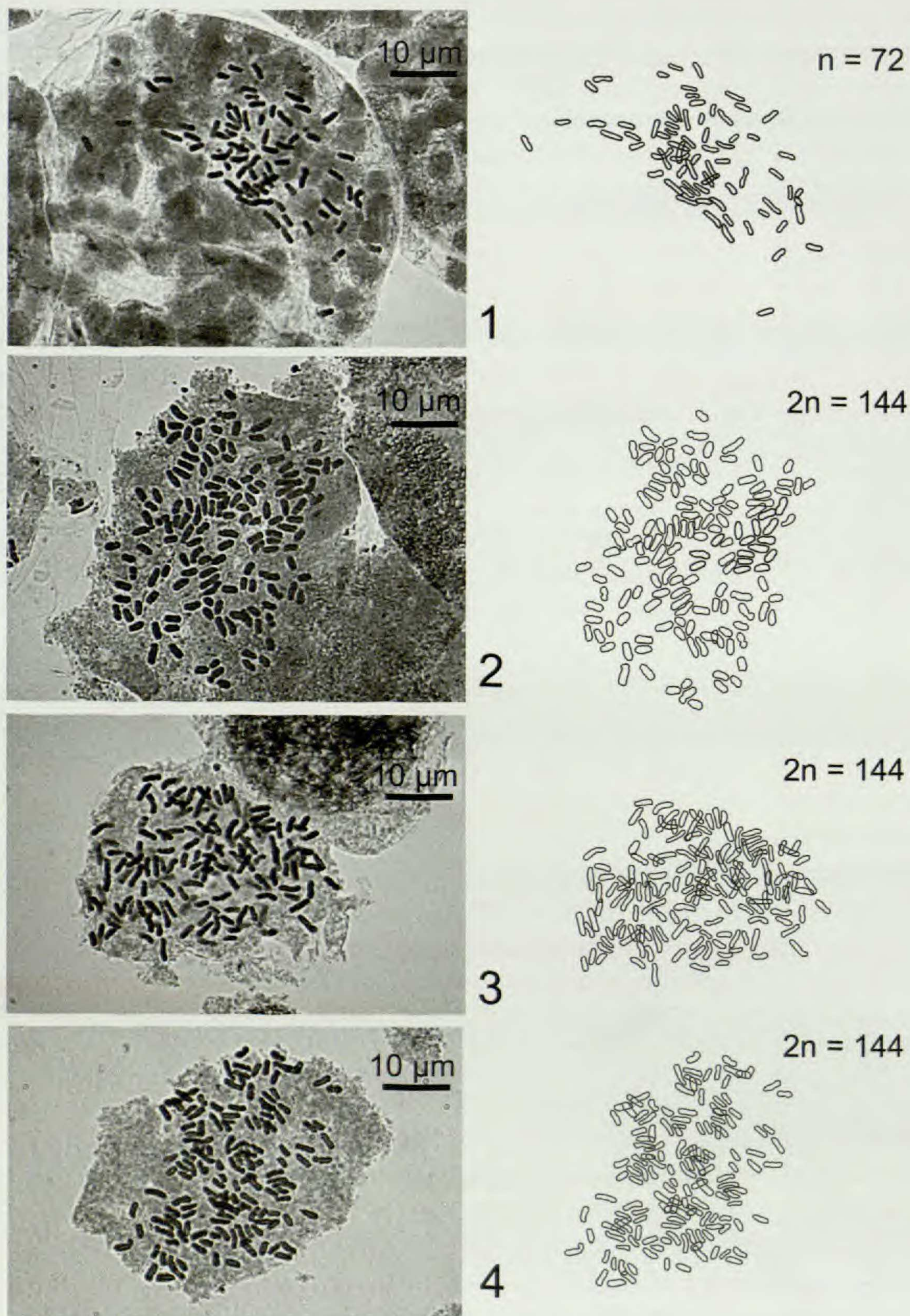
Diagnostic characters.—Stipe scales broadly lanceolate, $15\text{--}30 \times 2\text{--}6$ (8) mm; fronds narrowly lanceolate; midribs slightly prominent on both surfaces; sori long and sparse, occupying $2/3\text{--}3/4$ length of veins, 6–10 sori every 2 cm length along midribs.

Distribution.—China (Fujian), Japan (common in southern islands), Korea (Cheju Island). The misreport of this species in Hainan Island, China (Wu, 1999), is due to the misidentification of *A. phyllitidis* subsp. *malesicum* Holttum.

REPRESENTATIVES OF 32 SPECIMENS EXAMINED.—CHINA. **Fujian:** Luoyuan, $25^{\circ}07'N$, $123^{\circ}32'E$, 430 m, 12 Jul 2010, *Dong* 3403 (IBSC); without specific locality, 20 Aug 1943, *Lin* s.n. (IBSC, herb no. 295306). **Taiwan:** Nantou, 1300 m, 28 Aug 2002, *Lu* s.n. (PYU); Taitung, 23 Jan 1940, *Tagawa* 3071 (L).

KOREA. **Cheju:** without specific locality, 29 May 1908, *Taquet* 2369 (P).

JAPAN. **Hizen:** without specific locality, 16 Jun 1909, *Mistuo* s.n. (KYO). **Kagoshima:** Cape Sata, 19 Oct 1924, *Tashiro* s.n. (KYO); Oosumi, 26 Jul 1913,



FIGS. 1–4. Photomicrographs (left) and explanatory diagrams (right) of chromosomes at mitosis phase. 1. *Asplenium antiquum* cultivated in Guangzhou, China (Dong 3360); 2. *A. antrophyoides* from Guangxi, China (Dong 1976); 3. *A. australasicum* cultivated in Guangzhou, China (Dong 3400); 4. *A. cymbifolium* f. *lingganum* from Mindanao, Philippines (Dong 2610).

Tashiro s.n. (KYO); *ibid.*, 12 Aug 1916, *Tashiro s.n.* (KYO); *ibid.*, 100 m, 24 Jan 1965, *Togashi et al.* 10071 (L); Yakushima, Aug 1907, *Kudo* 5 (KYO); *ibid.*, Sep 1921, *Koidzumi s.n.* (KYO, PE); *ibid.*, 13 Dec 1956, *Togasi s.n.* (P); *ibid.*, 30–50 m, 14 Nov 1983, *Mitsuta & Nagamasu* 275 (PYU); *ibid.*, 30–100 m, 16 Jul 1979, *Yamazaki et al.* 2330 (KUN); 30 Jul 1961, *Ito* 289 (SING). **Nagasaki:** Naru, 28 Sep 1910, *Tashiro s.n.* (KYO). **Nagano:** without specific location, 1866–74, *Savatier* 1551 (P). **Okinawa:** Iriomote, 200 m, 19 Mar 1982, *Saiki* 2050 (PYU). **Wakayama:** Susami, 10 Aug 1913, *Ui s.n.* (KYO); Katsuura, 5 Jun 1951, *Kodama s.n.* (PE); Wakayama, Aug 1901, *Kinashi s.n.* (KYO). **Without specific locality:** 11 Nov 1881, *Dickins* 1877 (P).

In nomenclature, *Asplenium antiquum* was invalidly published by Makino (1929) because no type was designated. I tried to access the original material studied by Makino but failed. I designated a neotype for this species to validate the name *Asplenium antiquum* Makino.

Asplenium antrophyoides H. Christ in Bull. Acad. Int. Geogr. Bot. 20: 170. 1909. *Neottopteris antrophyoides* (H. Christ) Ching in Bull. Fan Mem. Inst. Biol., Bot. 10: 7. 1940. TYPE.—CHINA. Guizhou: Lofu, Sep 1907, *Cavalerie* 1877 (holotype, P!; isotype, BM!).

Cytology.—The somatic chromosome number is 144 for a population from Guangxi, China (Fig. 2). This is the first cytological record for *A. antrophyoides*.

Diagnostic characters.—Stipe scales broadly lanceolate, 15–20 × 3–5 mm; fronds more or less spatulate with the upper part the broadest; midribs obviously keeled on abaxial surface with the transection deltoid; sori long and usually sparse, occupying 2/3–3/4 length of veins, 7–10 (12) sori every 2 cm length along midribs.

Distribution.—China (Guangdong, Guangxi, Guizhou, Hunan, Sichuan, Yunnan), Thailand (Chiang Mai), Vietnam (Tonkin).

REPRESENTATIVES OF CA. 160 SPECIMENS EXAMINED.—CHINA. **Guangdong:** Liannan, 150 m, 16 Jan 2005, *Wang et al.* 725 (IBSC); Lianxian, 21 Nov 1930, *Ko* 50962 (PE). **Guangxi:** Huangjiang, 25°07'N, 107°58'E, 600 m, 13 Aug 2005, *Dong* 1976 (IBSC); Lingyun, 11 Dec 1933, *Steward & Cheo* 7 (PE); Longzhou (formerly Lungchow), 1901, *Morse* 63 (K); Napo, 23°00'N, 105°51'E, 1000 m, 13 Jun 2009, *Dong* 2956 (IBSC); Tianlin, 24°29'N, 106°21'E, 1150 m, 20 Jun 2009, *Dong* 3001 (IBSC). **Guizhou:** Libo, 25°17'N, 107°56'E, 600 m, 30 Jul 2010, *Dong et al.* GZE52 (IBSC); Pingchow, 350 m, 15 Sep 1930, *Tsiang* 7141 (BM, P). **Hunan:** Xinning, 370 m, 14 Nov 1962, *Liu* 15545 (IBSC, KUN). **Sichuan:** Qianwei, 380 m, 12 Aug 1984, *Xing et al.* 05235 (PE). **Yunnan:** Hekou, 22°40'N, 103°56'E, 150 m, 6 Oct 2009, *Song* 116 (IBSC); Jingdong, 1350 m, 7 Dec 1961, *Li* 3740 (KUN); Jinping, 850 m, 17 Jan 1959, *Chu* 4704 (PYU); Maguan, 650 m, 23 Nov 1982, *Chu et al.* 15340 (PYU); Mengla, 700–800 m, 25 Jul 1984, *Sino-Japan Exped.* 104 (KUN); Yanshan, 1100 m, 15 Nov 1939, *Wang* 84955 (KUN, PE).

THAILAND. **Chiang Mai:** Chiang Dao, 19°25'N, 98°55'E, 1500–1900 m, 7 Dec 1965, *Hennipman* 3256 (L); *ibid.*, 1600–2100 m, 4 Jan 1966, *Tagawa et al.* T4210 (IBSC, L); *ibid.*, 1600 m, 2 Mar 1995, *Maxwell* 95–176 (L)

VIETNAM. **Cao Bang:** Nguyen Binh, 22°39'N, 105°57'E, 500–550 m, 21 Apr 1999, *Loc et al.* 1609 (P); without specific locality, Feb 1925, *Colani* 1982 (P). **Lai Chau:** Ngai Thau, 1000–1200 m, 7 Apr 1936, *Poilane* 25554 (P); without specific locality, 800–900 m, 6 Jan 1938, *Poilane* 27030 (P). **Thanhhoa:** Lung Van, 1000–1200 m, 26 Jan 1931, *Poilane* 18889 (P).

Asplenium australasicum (J. Sm) Hook., *Fil. Exot.* t. 88. 1859. *Neottopteris australasica* J. Sm., *Cult. Ferns* 49. 1857. TYPE.—*Cult. Hort. Bot. Kew.*, origin Australia (lectotype designated by Holttum in 1974, BM? isotype, K!).

Cytology.—Cultivated plants from South China Botanical Garden were examined. A total of 144 chromosomes were counted for sporophytes (Fig. 3) and about 72 for gametophytes. Before my examination, this species was reported to be tetraploid with 144 chromosomes based on root-tips from Middle Brother and Lord Howe Island, Australia (Tindale and Roy, 2002).

Diagnostic characters.—Stipe scales narrowly lanceolate, 10–20 × 1–1.5 mm; fronds lanceolate; midribs distinctly keeled on abaxial surface with the transection deltoid; sori short and dense, occupying less than 1/2 length of veins, (10)13–18 sori every 2 cm length along midribs.

Distribution.—Australia (Queensland, New South Wales, Lord Howe Island), Fiji, Samoa, Tonga, Vanuatu (New Hebrides), Polynesia (Tahiti) and other Pacific islands.

REPRESENTATIVES OF CA. 110 SPECIMENS EXAMINED.—AUSTRALIA. **Lord Howe Island:** without specific locality, Sep 1853, *Herald* 696 (K); *ibid.*, Dec 1869 (?), *More* 76 (K). **New South Wales:** Coffs Harbour, 150 m, 2 May 1956, *Constable* P7404 (K); Newell Falls Creek, 3000 ft, *Werner* 18 (K). **Queensland:** Cape York, 420 m, 17 Aug 1948, *Brass* 19879 (K); Mt Lewis, 16°29'S, 145°14'W, 21 Sep 1975, *Coveny* 7242 (K, L); Mt Mistake, 24 Nov 1930, *Hubbard* 5202 (K); Richmond River, 1885, *Anonymous s.n.* (SING, herb no. 0079510).

PACIFIC ISLANDS. **Christmas:** 1°52'N, 157°20'W, 4 Dec 1987, *Maclean s.n.* (K). **Fiji:** Ovalau, 17°42'S, 178°48'W, 100–300 m, 8–11 May 1953, *Smith* 7322 (K, L); Viti Levu, 17°58'S, 178°06'W, 150–250 m, 17 Oct 1953, *Smith* 8602 (K, L). **Guam:** Finegayan, 29 Jul 1987, *Flis* 15398 (K). **Henderson:** North end, 24°22'S, 128°19'W, 5 m, 17 June 1934, *John & Fosberg* 15088 (K). **Mangaia:** 21°55'S, 157°55'W, Oct 1972, *Dickie* 25 (K). **New Caledonia:** 21°26'S, 165°54'W, Nov 1911, *Anonymous* 147 (K); without specific locality, Mar 1889, *Hennecart s.n.* (K). **Niue:** 19°01'S, 169°55'W, 20 m, 11 Jan 1940, *Yuncher* 9590 (K). **Oeno:** 23°56'S, 130°44'W, 1921(?), *Quayle* 409 (K). **Samoa:** Laugapapa, Sep 1877, *Powell* 164 (K); without specific locality, Jun 1872(?), *Powell* 164 (K). **Tahiti:** Mt Marau, 17°37'S, 149°32'W, 1250 m, 30 May 1985, *Fosberg* 64558 (K). **Tonga:** Tavau, 18°40'S, 174°00'W, *Crosby* 277 (K). **Vanuatu:** New Hebrides, 17°31'S, 168°21'W, 580 ft, 2 Jul 1971, *Braithwaite* 2002 (K).

This species is morphologically characterized by having fronds ascending steeply and midribs distinctly keeled on abaxial surface. Nakato (1987) reported a population of “*A. australasicum*” from Iriomotejima, Japan, with 144 somatic chromosomes. However, *A. australasicum* is known so far to be restricted to Queensland and nearby Pacific islands. The so-called *A.*

australasicum from southern Japan proved to be *A. setoi* N. Murak. & Seriz. based on molecular data (Murakami *et al.*, 1999).

Asplenium cymbifolium* f. *lingganum Alderw. in Bull. Jard. Bot. Buitenzorg III, 5: 184. 1922. TYPE.—INDONESIA. Lingga Arch., 8 Aug 1919, *Bunnemeijer* 7388 (holotype, K!, isotype, L).

Cytology.—Both the root tips and prothalli originally from a Philippine population were cytologically examined. The chromosome number is 144 for root tips (Fig. 4) and ca. 72 for young prothalli. This taxon is confirmed to be a sexual tetraploid for the first time.

Diagnostic characters.—Stipe scales narrowly lanceolate, ca. $10 \times 1-2$ mm; fronds lanceolate and widened at base; midribs prominent on abaxial surface with the transection elliptic; sori long and sparse, occupying $2/3-3/4$ length of veins, 4–7 (9) sori every 2 cm length along midribs.

Distribution.—Indonesia (Lingga Arch., Sumatra), Papua New Guinea, Philippines (Mindanao).

ADDITIONAL SPECIMENS EXAMINED.—INDONESIA. **Jappen-Biak**: Wasabori near Seroei, 12 Aug 1939, *Aet & Idjam* 400 (BO). **Sumatra**: Sikundar Nature Conservations, 100–250 m, 14 Apr 1971, *Iwatsuki et al.* S361 (K). **Ditschi**: without specific locality, 1200 m, Jun 1928, *Mayr* 183a (BO).

PAPUA NEW GUINEA. **East Sepik**: Mt Samai, $4^{\circ}29'S$, $142^{\circ}41'E$, 450 m, 27 Apr 1991, *Takeuchi* 6216A (K).

PHILIPPINES. **Mindanao**: Marilog, Davao, 1200 m, 17 Nov 2007, *Dong* 2610 (IBSC).

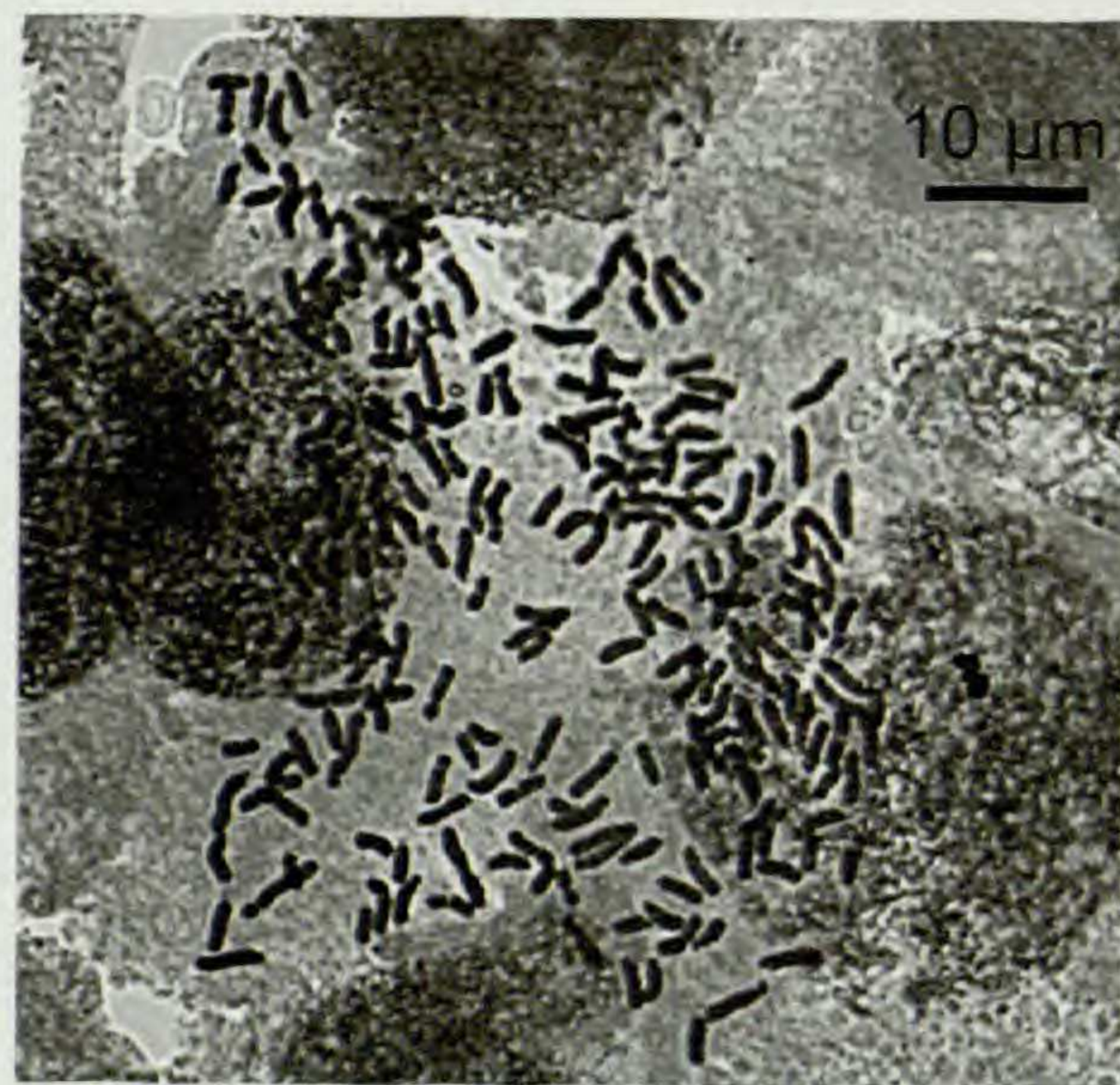
In comparison with *A. cymbifolium* with wide fronds (18–20 cm), the form *lingganum* was characterized by much smaller fronds (6 cm wide). Besides the remarkable difference in frond size the form *lingganum* differs from the form *cymbifolium* in lacking dense scales on abaxial surface of midrib towards the base. So it seems more suitable to treat the form *lingganum* as a variety or a separate species from *A. cymbifolium*.

Asplenium humbertii Tardieu, *Asplen. Tonkin* 25, pl. 2, f. 1–2. 1932. *Neottopteris humbertii* (Tardieu) Tagawa in J. Jap. Bot. 22: 161. 1948. TYPE.—VIETNAM. Tonkin, Jan 1886, *Balansa* 68 (holotype, P!).

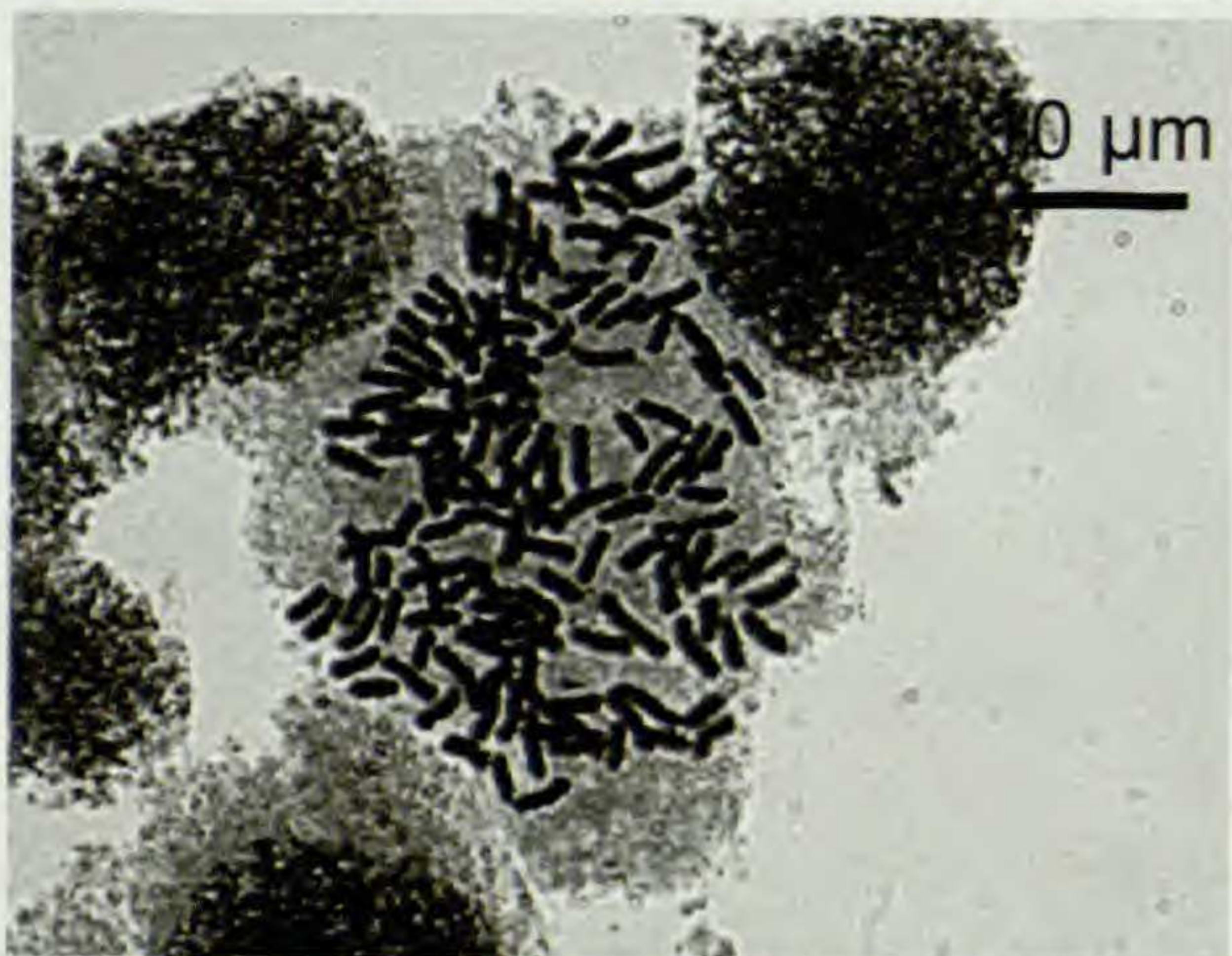
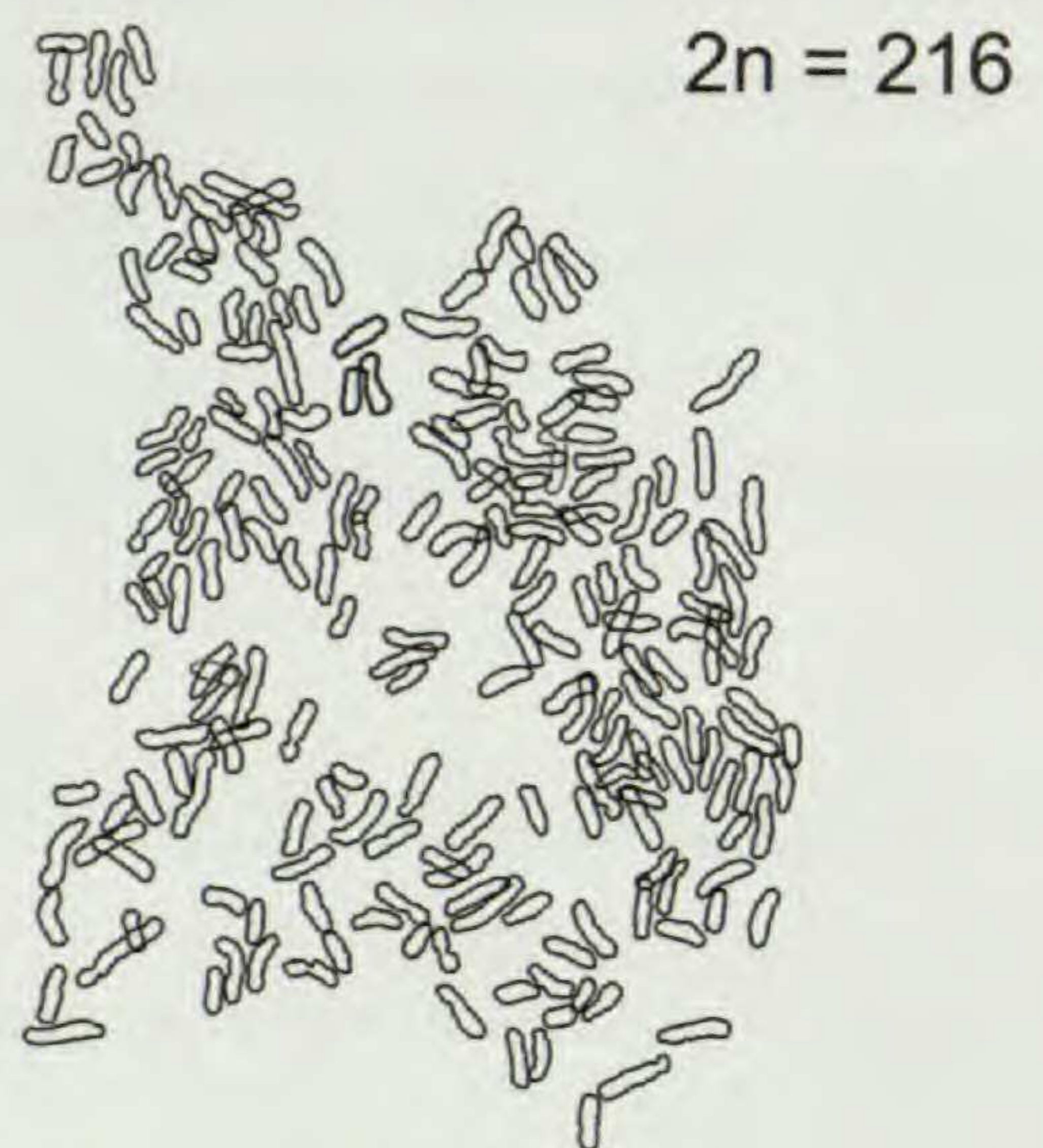
Cytology.—A population from Hainan Island and other one from Guangxi, China proved to be sexual hexaploids with chromosome number $2n = 216$ (Fig. 5). This species is the only hexaploid so far found in *Asplenium* sect. *Thamnopteris*.

Diagnostic characters.—Stipe scales broadly lanceolate, $8-10 \times 2-3$ mm; fronds more or less spatulate with long and narrow stipes; midribs slightly prominent with the transection elliptic; sori long and usually sparse, occupying more than $2/3$ length of veins, 8–13 sori every 2 cm length along midribs.

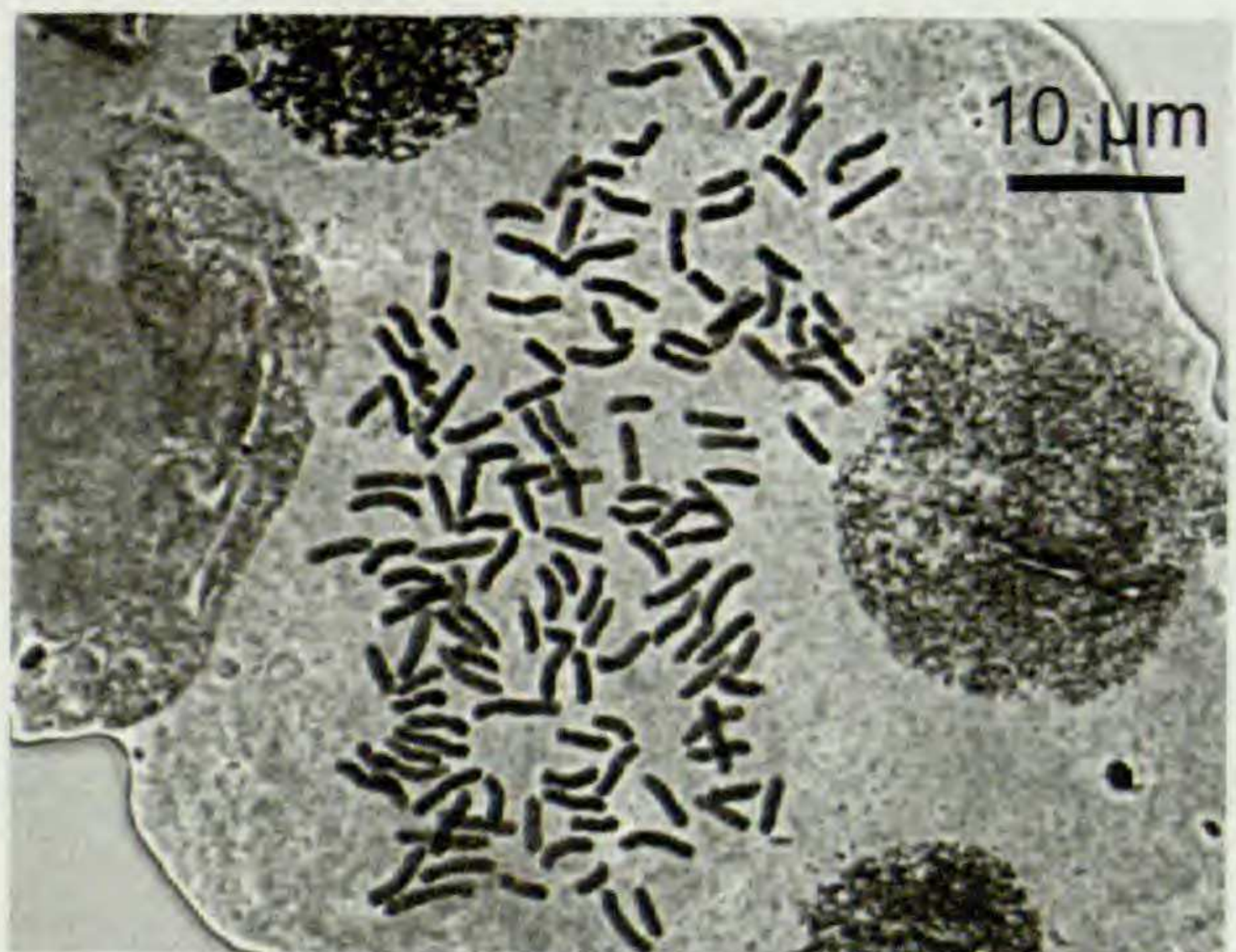
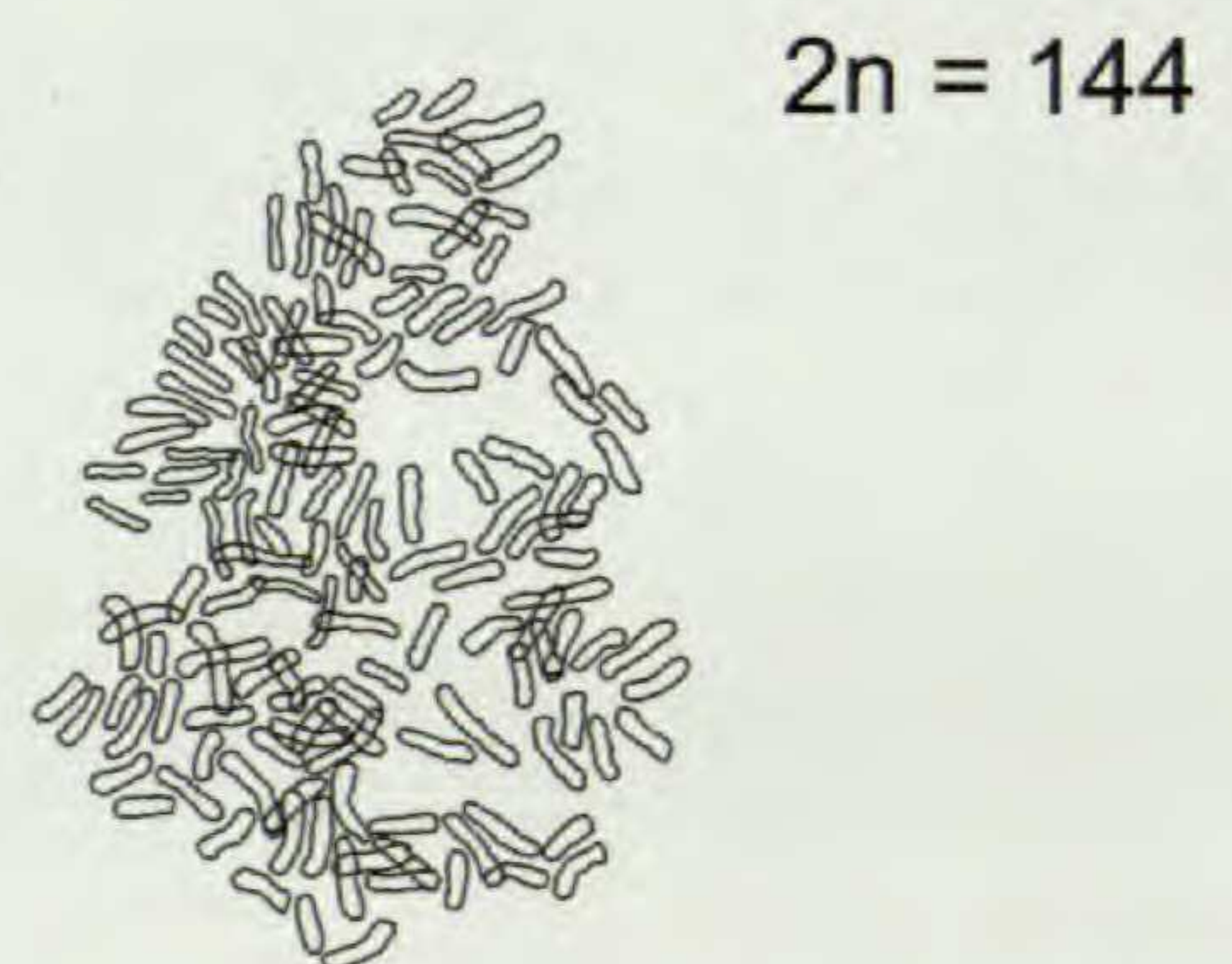
Distribution.—China (Hainan, Guangxi, Yunnan), Laos (Cammon), Thailand (Chiang Mai), Vietnam (Tonkin). *Asplenium humbertii* only occurs in limestone within these regions.



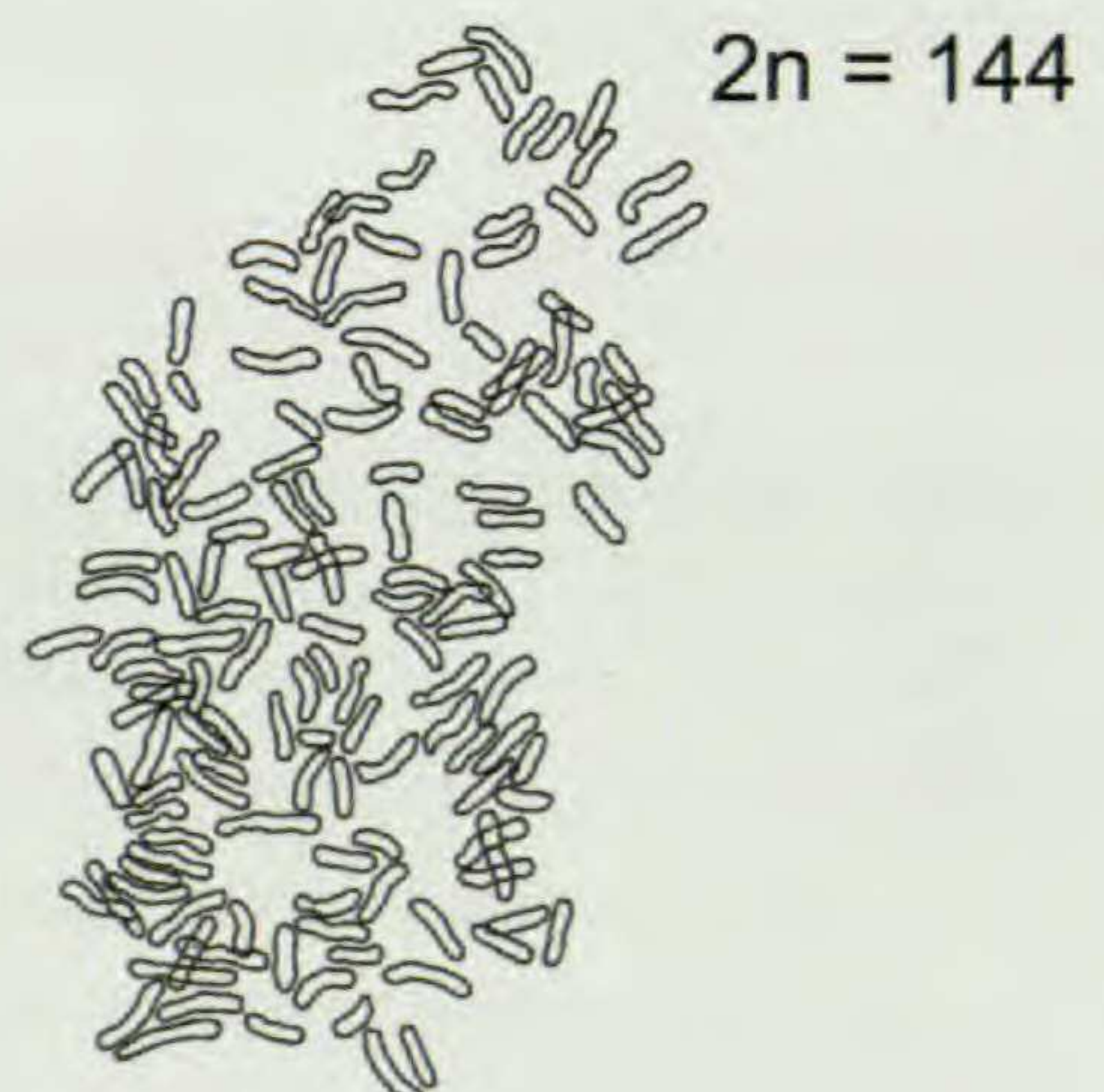
5



6



7



FIGS. 5–7. Photomicrographs (left) and explanatory diagrams (right) of chromosomes at mitosis phase. 5. *Asplenium humbertii* from Guangxi, China (Dong 2287B); 6. *A. nidus* from Guangxi, China (Dong 2939); 7. *A. phyllitidis* subsp. *malesicum* from Hainan, China (Dong 1645).

ADDITIONAL SPECIMENS EXAMINED.—CHINA. **Hainan**: Changjiang, 19°01'N, 109°06'E, 1000 m, 20 Oct 2004, *Dong 1234* (IBSC); *ibid.* 5 Apr 1987, *Chen 703* (IBSC). **Guangxi**: Longzhou, 22°34'N, 106°48'E, 200 m, 14 Mar 2007, *Dong 2287B* (IBSC). **Yunnan**: Hekou, 22°40'N, 103°56'E, 150 m, 11 Aug 2010, *Dong 3248* (IBSC); *ibid.*, 400 m, *Song 119* (IBSC); *ibid.*, 400 m, Feb 1959, *Chu 3922* (PYU). Jinping, 23°02'N, 103°24'E, 500 m, 12 Aug 2010, *Dong 3432* (IBSC); *ibid.*, 600 m, 10 Oct 2009, *Song 133* (IBSC).

LAOS. **Cammon**: Tham, 160 m, Nov 1930, *Colani 4098* (P); Thok, 160 m, Nov 1930, *Colani 4091* (P).

THAILAND. **Chiang Mai**: Chiang Dao, 1100 m, 16 Feb 1958/59, *Sorensen et al.* 1237 (K); *ibid.*, 550 m, 15 Jan 1989, *Maxwell 89-59* (L). **Chiang Rai**: local name unknown, 500 m, 27 May 1926, *Garrett 289* (K). **Muang Sing**: Kwai Noi Basin, 150 m, 3 Jun 1946, *Hoed 920* (L).

VIETNAM. **Bac Giang**: Lang Met, May 1929, *Colani 1982* (P). **Lang Son**: Lang Mac, Feb 1929, *Colani s.n.* (P). Without specific locality, 260–390 m, 22 Dec 1964, *Sino-Vietnam Exped. 699* (PE).

Asplenium nidus L., Sp. Pl. 1079. 1753. *Neottopteris nidus* (L.) J. Sm. in J. Bot. 3: 409. 1841. TYPE.—INDONESIA. Java, *Osbeck 49* (lectotype designated by Holttum in 1974, L).

Cytology.—Wild plants of this species from Hainan, Yunnan, Guangxi, or cultivated plants (Table 1) proved to be sexual tetraploid with chromosome number $2n = 144$ (Fig. 6) or $n = 72$. This species has been reported as tetraploid from northern India (Bir, 1960), southern India (Abraham *et al.*, 1962), Kagoshima, Japan (Kawakami, 1970, 1997), Taiwan (Tsai and Hsieh, 1983), Hainan Island, China (Kato and Nakato, 1999), and from Java, Indonesia (Yatabe *et al.*, 2001).

Diagnostic characters.—Stipe scales narrowly lanceolate, $15\text{--}20 \times 1\text{--}2$ mm; fronds narrowly lanceolate; midribs obviously prominent on adaxial surface and nearly flat on abaxial surface; sori short and dense, occupying usually less than 1/2 length of veins, (10) 12–18 sori every 2 cm length along midribs.

Distribution.—The whole range of *Asplenium* sect. *Thamnopteris*, mainly on tropical Asia and Pacific islands, west to Africa and northeast to Hawaii.

REPRESENTATIVES OF CA. 1220 SPECIMENS EXAMINED.—AUSTRALIA. **Queensland**: Iron Range, Cape York Peninsula, 20 m, 6 Jun 1948, *Brass 19049* (K).

CHINA. **Xizang**: Motuo, 800 m, 11 Aug 1974, *Qinghai-Xizang Exped. 74-4215* (KUN, PE). **Hainan**: Mt Yinggeling, 680 m, 3 Dec 2005, *Dong 1689* (IBSC). **Guangxi**: Napo, 23°00'N, 105°51'E, 1000 m, 13 Jun 2009, *Dong 2939* (IBSC). **Yunnan**: Malipo, 23°01'N, 104°46'E, 780 m, 17 Jun 2009, *Dong 2980* (IBSC).

INDIA. **Andamans**: Mt Harriet, 3 Jan 1998, *Balachandra 0879* (K).

INDONESIA. **Kalimantan**: Balikpapan, 01°09' S, 116°50' E, 40 m, 16 Dec 2010, *Dong 3470* (IBSC); Samarinda, 01°07' S, 117°12' E, 20 m, 18 Dec 2010, *Dong 3474* (IBSC). **Irian Jaya**: Manokwari, 00°59' S, 133°58' E, 40 m, 12 Dec 2010, *Dong 3455 & 3456* (IBSC). **Java**: Bogor, 280 m, 25 Oct 2009, *Dong 3362* (IBSC). **Seram**: Manusela National Park, 3°08–09'S, 129°29'E, 1290–2000 m, 3 Jan 1985, *Kato et al. C350bis* (BO).

JAPAN. **Bonin**: Hahajima, 3 Jun 1971, *Sohma et al.* 715352 (KYO); **Kagoshima**: Yakushima, 12 Aug 1957, *Iwatsuki* 3285 (KYO).

KENYA. **Kwale**: Kaya Chombo, 04°08'S, 39°29'E, 240 m, 1 Sep 1999, *Luke & Mbinda* 5972 (K).

MALASIA. **Malay Peninsula**: Perak, 2500–3000 ft, Jul 1884, *King's Collector* 6347 (SING).

MYANMAR. **Bhamo**: Palin to Nampa, 500 ft, 15 Dec 1908, *Lace* 4495 (K).

PACIFIC ISLANDS. **Hawaii**: Wai'anae Mts, O'ahu, 21°33'N, 158°11'W, 1550 ft, 11 Aug 1973, *Herbst* 3090 (K). **Tonga**: Eua Island, 21°22'S, 174°56'W, 200–300 m, Jun 1926, *Parks* 16258 (K). **Vanuatu**: Tegua, 220 m, 28 Oct 1993, *Curry* 1364 (K).

PHILIPPINES. **Luzon**: Mt Sicapoo, 800 m, 6 Dec 1975, *Iwatsuki et al.* P890 (K).

SIKKIM. **Ratongchu**, 1000 m, 15 May 1960, *Hara et al.* 2416 (BM, K).

SRI LANKA. **Sabaragamuwa**: Ratnapura, 6°35'N, 80°43'E, 580–600 m, 30 Dec 1976, *R.B. & Faden* 653 (K). **Opanake**: without specific locality, 3 Jan 1951, *Ballard* 1372 (K).

THAILAND. **Udawn**: Mt Luang, 17°25'N, 101°25'E, 750 m, 7 Jan 1966, *Hennipman* 3522 (BM).

UKUNDA. **Longomwagandi**: Shimb Hills, 04°14'S, 39°25'E, 380 m, 12 Jun 1996, *Luke et al.* 4512 (K).

VIETNAM. **Bac Can**: Na Ri, 22°17'S, 106°03'E, 550–600 m, 20 Oct 1999, *Hiep et al.* 3746 (K).

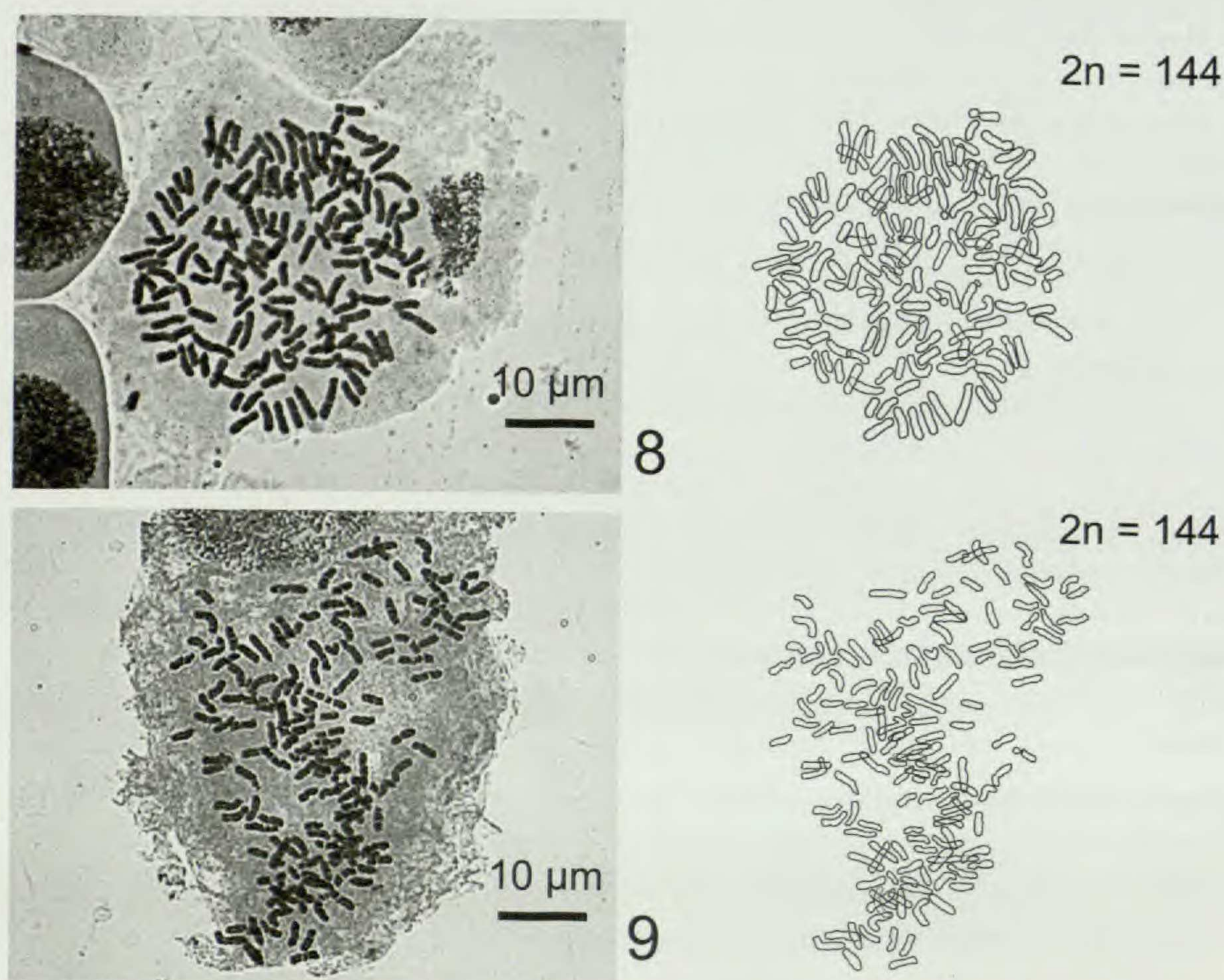
Molecular data indicate *A. nidus* is a complex containing several cryptic species (Murakami *et al.*, 1999; Yatabe *et al.*, 2001). The data of spore morphology have showed that the perispores are fenestrate and alately folded in populations of *A. nidus* from South China but imperforate and costately folded in those from Java, Indonesia (pers. obs.), which supports the *A. nidus* in present description is a complex. A taxonomic treatment on this complex is needed.

Asplenium phyllitidis* subsp. *malesicum Holttum in Gard. Bull. Singapore 27: 153. 1974. TYPE.—PHILIPPINES. Samar, *Cuming* 319 (holotype, K!).

Cytology.—Both root tips and young prothalli were examined for a population from Hainan Island, China (Fig. 7), and one from Mindanao, Philippines. The chromosome number, $2n = 144$ and $n = \text{ca. } 72$, were found in both populations. In addition, a cultivated plant with the origin unknown was confirmed to be a tetraploid (Fig. 8). This is the first cytological examination for this taxon.

Diagnostic characters.—Stipe scales narrowly lanceolate, $10\text{--}15 \times 1\text{--}2$ mm; fronds narrowly lanceolate; midribs obviously prominent on abaxial surface with the transection bluntly deltoid; sori long and sparse, occupying usually more than $3/4$ length of veins, 7–9 sori every 2 cm length along midribs.

Distribution.—Throughout Malesia (Singapore, Malaysia, Indonesia, New Guinea, Philippines, Caroline Islands), Society Islands, Indo-China Peninsular (Thailand, Vietnam), southwestern China (including Hainan Island).



FIGS. 8–9. Photomicrographs (left) and explanatory diagrams (right) of chromosomes at mitosis phase. 8. *Asplenium phyllitidis* subsp. *malesicum* cultivated in Guangzhou, China (Dong 3360); 9. *A. simonsianum* from Yunnan, China (Dong 2768).

REPRESENTATIVES OF CA. 600 SPECIMENS EXAMINED.—CHINA. **Guangxi:** Tianlin, 16 Jun 1958, *Li* 600812 (IBSC, KUN). **Hainan:** Mt Yinggeling, 18°57'N, 109°24', 1170 m, 27 Nov 2005, *Dong* 1625 (IBSC). **Yunnan:** Mengla, 21°36'N, 101°35', 700 m, 28 Apr 2008, *Dong* 2762 (IBSC); Pingbian, 1400 m, 17 Jun 1934, *Tsai* 60226 (KUN, P, PE).

INDONESIA. **Kalimantan:** Balikpapan, 01°09'S, 116°50'E, 40 m, 16 Dec 2010, *Dong* 3469 (IBSC). **Sumatra:** Asahan, Nov 1932, *Krukoff* 4305 (SING). **Ternate:** Castela, 4 Aug 1954, *Alston* 16661 (BO). **Java:** Bodogol, 06°46'S, 106°51'E, 820 m, 1 Nov 2009, *Dong* 3384 (IBSC).

MALAYSIA. **Pahang:** Kuantan, 130 m, 6 Jun 1968, *Ogata* 10469 (L). **Sarawak:** Pulan Bruit, 12 ft, 14 Jun 1957, *Anderson* 7939 (L).

PACIFIC ISLANDS. **Bismarck:** Mandiuh Lake, Namatanai, 4°28'S, 153°3'E, 650 m, 7 Oct 1975, *Croft* 68298 (L). **Caroline:** Urukthapel, 1–10 m, 17–19 Mar 1950, *Fosberg* (L). **New Britain:** Talasea, 5°21'S, 149°58'E, 600 m, 23 Oct 1974, *Barker & Vinas* 66659 (L). **New Ireland:** Tamai River, 600 m, 14 Oct 1975, *Croft* 254 (L). **Society:** Guadalcanal, 2000 ft, 5 Nov 1962, *Whitmore & Womersley* 1045 (L); Raiatea Island, 160 m, 5 Oct 1926, *Moore* 171A (L).

PAPUA NEW GUINEA. **Kaisenik**, 07°20' S, 146°40' E, 2200 m, 17 Feb 1978, *Unkau 025* (K, L). **Maneau**: Mt Dayman, Maneau, 16 Jul 1953, *Brass 23439* (L).

PHILIPPINES. **Laguna**: Los Banos, 14°08'N, 121°12'E, Jun–Jul 1917, *Elmer 17976* (K). **Leyte**: Palo, 11°10'N, 124°59'E, Jan 1906, *Elmer 7037* (K). **Mindanao**: Davao, 1200 m, 16 Nov 2007, *Dong 2606* (IBSC). **Mindoro**: Luzon, 13°17'N, 121°00'E, 1200 m, 15 Mar 1997, *Argent et al. 20104* (L). **Sorsogon**: Irosin, 12°46'N, 124°03'E, Sep 1916, *Elmer 17312* K).

THAILAND. **Trat**: Klawng Mayom, 100 m, 16 Feb 1955, *T.S. 2177* (L). **Narathiwat**: Tak Bai, 1 Sep 1987, *Niyomdham & Sriboonma 1475* (K).

VIETNAM. **Quang Ninh**: Mong Cai, 27–30 Sep 1936, *Tsang 26918* (IBSC, K).

This taxon is very widespread in forest area of tropical Asia and Pacific islands but herbarium materials are too often misidentified as *A. phyllitidis* subsp. *phyllitidis* or *A. nidus*. As being distinct from other members of *Thamnopteris* in bluntly deltoid midribs in transection, long and sparse sori, and exclusively spinulate perispores, *A. phyllitidis* subsp. *malesicum* is suggested to be a separate species but not a subspecies. A detailed discussion and taxonomic treatment are being prepared by the present author in another paper.

Asplenium simonsianum Hook., *Icon. Pl.* 10: t. 925. 1854. *Neottopteris simonsiana* (Hook.) J. Sm., *Hist. Fil.* 330. 1875. TYPE.—INDIA. Khasia, *Simons 232* (err. 432) (holotype, K!).

Cytology.—The chromosome number carried out to be $2n = 144$ for a population of this species from Yunnan, China for the first time (Fig. 9). It is a sexual tetraploid.

Diagnostic characters.—Stipe scales ovate or broadly lanceolate, $2\text{--}3 \times 1$ mm or $5\text{--}8 \times 1.5\text{--}2$ mm; fronds narrowly lanceolate; midribs slightly prominent on both surfaces; sori long, dense or sometimes sparse, occupying usually $2/3\text{--}3/4$ length of veins, 10–13 sori every 2 cm length along midribs.

Distribution.—Southwestern China (Yunnan, Xizang), northeastern India (Khasia, Assam), Nepal, Bhutan, Sikkim, Myanmar, Thailand (Lampang).

REPRESENTATIVES OF CA. 160 SPECIMENS EXAMINED.—BANGLADESH. **Sylhet**, *Wallich 723* (BM). “Ham. Hanopokhri”, 18 Jul 1914, *Ghose 11* (P).

BHUTAN. **Gelephu** (formly Surelakha), 4000 ft, 1 Oct 1937, *Ludlow & Sherriff 2932* (BM); *ibid.*, 3000 ft, 29 Mar 1949, *Ludlow et al. 18727* (BM).

CHINA. **Guangxi**: Fusui, 350 m, 26 Apr 1957, *Chun 12136* (IBSC, KUN, PE). **Yunnan**: Fohai, 25 Jun 1956, *Yunnan Univ Exped. 579* (PE); Jinghong, 960 m, Jan 1958, *Yunnan Univ Exped. 682* (PE); Jinping, 27 Apr 1956, *Sino-Russia Exped. 302* (IBSC, PE); Lvchun, 1800 m, 11 Jul 1973, *Tao 742* (IBSC, KUN); Maguan, 19 Feb 1990, *Min 276* (KUN); Mengla, 21°36'N, 101°35'E, 700 m, 29 Apr 2008, *Dong 2768* (IBSC); Mengzi, Apr 1953, *Cai 280* (PE); Nabang, 8 Sep 1980, *Dong 792* (PE). **Xizang**: Motuo, 19 Aug 1980, *Chen 14290* (PE); unknown, 28°00'N, 97°45'E, 4000 ft, 1926, *Ward s.n.* (K).

INDIA. **Assam**: Digboi, 450–700 ft, 2 Mar 1936, *Barnard 43A* (BM); Garrow Hills, 1880, *Day s.n.* (K); Gauhati Hills, *Simons s.n.* (BM); Naga Hills, 5000 ft, 8

Feb 1946, *Bor* 44 (BM). **West Bengal:** Darjeeling, 1871, *Anonymous* s.n. (P, "Syn. Fil. no. 1").

NEPAL. **Pokhara:** Bakhri Kharka, 4500 ft, 23 Apr 1954, *Stainton et al.* 5005 (BM).

THAILAND. **Lampang:** without specific locality, 450 m, *Smith(?)* 895 or 1682 (K). **Kamphaeng Phet:** Hual Krasa, 16°05'N, 99°09'E, 900 m, 19 Mar 1968, *Hansen & Smitinand* 12972 (K). **Phetchabun:** Phu Miang, 1200–1300 m, 2 Oct 1967, *Shimizu et al.* T11378 (L).

KEY TO THE SPECIES AND INTRASPECIFIC TAXA OF *ASPLENIUM* SECT.

THAMNOPTERIS RECORDED HERE

1. Stipe scales narrowly lanceolate, the ratio of length to width more than 10
 2. Frond midribs flat or slightly rounded on abaxial side *A. nidus*
 2. Frond midribs obviously prominent, keeled or nearly so on abaxial side
 3. Frond midribs not typically keeled on abaxial side, bluntly triangular in transection; perispores echinate *A. phyllitidis* subsp. *malesicum*
 3. Frond midribs strongly keeled on abaxial side, sharply triangular in transection; perispores folded *A. australasicum*
1. Stipe scales ovate or broadly lanceolate, the ratio of length to width usually being 2 to 5
 4. Frond midribs strongly keeled on abaxial side, sharply triangular in transection *A. antrophyoides*
 4. Frond midribs rounded on abaxial side, ellipse or nearly so in transection
 5. Fronds rounded at base; wingless stipes lacking *A. cymbifolium* f. *lingganum*
 5. Fronds cuneate at base; wingless stipes present
 6. Stipes with very narrow wings along either side, the same long or a bit shorter than laminae; chromosome number $2n = 216$; restricted to limestone area. *A. humbertii*
 6. Stipes very short, less than 1/20 the length of laminae; chromosome number $2n = 144$; not occurring on limestone area
 7. Fronds 8–12 cm wide; stipe scales 15–30 mm long *A. antiquum*
 7. Fronds 3–6 cm wide; stipe scales less than 8 mm long *A. simonsianum*

PHYLOGENETIC SIGNIFICANCE OF THE CYTOLOGICAL DATA

The eight species recorded here are widely accepted species which constitute the majority of *Asplenium* sect. *Thamnopteris*. Observations in this study and those by previous authors (Bir, 1960; Abraham *et al.*, 1962; Kawakami, 1970, 1997; Kato and Nakato, 1999; Yatabe *et al.*, 2001; Tindale and Roy, 2002) showed that the major members of *Thamnopteris*, several with more than one population from various regions examined, are sexual tetraploids and *A. humbertii* is the only hexaploid so far found. It is readily presumed that these tetraploids originated from diploids via some genetic mechanism which resulted in doubled chromosome sets. *Asplenium humbertii* with six chromosome sets is presumed to have a hybrid origin in that a diploid ancestor crossed with a tetraploid and subsequently, the triploid hybrid changed to sexual by doubling chromosome sets. Before this study, no diploid species was known in the section *Thamnopteris*, which suggests this group probably originated from a diploid *Asplenium* species outside of section *Thamnopteris*.

ACKNOWLEDGMENTS

I sincerely thank the staff of the following herbaria for providing research facilities: BO, BM, GAUA, HITBC, IBK, IBSC, K, KUN, L, P, PE, PNH, and PYU. I owe debts of gratitude to curators and staff of KYO and SING for sending loans of specimens to IBSC. My gratitude also goes to two anonymous reviewers and Jennifer Geiger for their valuable comments. This work was supported by the National Nature Science Foundation of China (grant no. 30770163), Knowledge Innovation Program of the Chinese Academy of Sciences (grant no. KSCX2-YW-Z-0917), and Guangzhou Science and Technology Asian Games Special Action Plan (2010U1-E00601).

LITERATURE CITED

- ABRAHAM, A., C. A. NINAN and P. M. MATHEW. 1962. Studies on the cytology and phylogeny of the pteridophytes. VII. Observations on one hundred species of south Indian ferns. *J. Indian Bot. Soc.* 41:339–421.
- BELLEFROID, E., S. K. RAMBE, O. LEROUX and R. L. L. VIANE. 2010. The base number of 'loxoscapoid' *Asplenium* species and its implication for cytoevolution in Aspleniaceae. *Ann. Bot.* 106:157–171.
- BIR, S. S. 1960. Cytological observations on the East Himalayan member of *Asplenium* L. *Curr. Sci.* 29:445–447.
- CHING, R. C. and C. H. WANG. 1964. Additional materials for the pteridophytic flora of Hainan. *Acta Phytotax. Sin.* 9:345–373.
- HOLTUM, R. E. 1974. *Asplenium* L. sect. *Thamnopteris* Presl. *Gard. Bull. Singapore* 27:143–154.
- JONES, D. L. 1988. New fern species from northern Australia. *Austrobailey* 2:469–480.
- KATO, M. and N. NAKATO. 1999. A cytotaxonomic study of Hainan (S China) pteridophytes with notes on polypoidy and apogamy of Chinese species. Pp. 1–19, *In*: X. C. Zhang and K. H. Shing, eds. *Ching Memorial Volume*. Beijing: Chinese Forestry Publishing House.
- KAWAKAMI, S. 1970. Karyological studies on Aspleniaceae II. *Bot. Mag. (Tokyo)* 83:74–81.
- KAWAKAMI, S. 1997. Induction of apogamy in twelve fern species and the study of their somatic chromosomes. *Chromosome Science* 1:89–96.
- KRAMER, K. U. and R. L. L. VIANE. 1990. Aspleniaceae. Pp. 52–56, *In*: K. U. Kramer and P. S. Green, eds. *Pteridophytes and gymnosperms*. Berlin: Springer-Verlag.
- LINNAEUS, C. 1753. *Species Plantarum*, Stockholm.
- LOVIS, J. D. 1977. Evolutionary patterns and processes in ferns. *Adv. Bot. Res.* 4:229–451.
- MAKINO, T. 1929. A contribution to the knowledge of the Flora of Japan. *J. Jap. Bot.* 6:29–32.
- MANTON, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.
- MIAO, R. H. 1980. Additions to the pteridophytic flora of Hainan. *Acta Sci. Nat. Univ. Sunyatseni* 1:99–100.
- MURAKAMI, N., M. WATANABE, J. YOKOYAMA, Y. YATABE, H. IWASAKI and S. SERIZAWA. 1999. Molecular taxonomic study and revision of the three Japanese species of *Asplenium* sect. *Thamnopteris*. *J. Pl. Res.* 112:15–25.
- NAKATO, N. 1987. Notes on chromosomes of Japanese pteridophytes (1). *J. Jap. Bot.* 62:261–267.
- PRESL, C. B. 1836. *Tentamen Pteridographiae, seu Genera Filicacearum*. Typis Filiorum Theophili Haase, Prague.
- SETO, K. 1979. Notes on the bird's nest ferns in Japan. *Nankiseibutu* 21:6–10.
- SMITH, J. 1841. *Genera Filicum*, an arrangement and definition of the genera of ferns with observations on the affinities of each genus. *Hooker's J. Bot. Kew Gard. Misc.* 3:409.
- TARDIEU-BLOT, M. 1933. Contribution a l'étude des Aspléniées de l'Ido-Chine I, *Asplenium*. *Bull. Mus. Natl. Hist. Nat.* 5:480–487.
- TINDALE, M. D. and S. K. ROY. 2002. A cytotaxonomic survey of the Pteridophyta of Australia. *Aus. Sys. Bot.* 15:839–937.
- TSAI, J. L. and W. Q. SHIEH. 1983. A cytotaxonomic survey of the pteridophytes in Taiwan. *J. Sci. Engin.* 20:137–158.

- WALKER, T. G. 1979. The cytogenetics of ferns. Pp. 87–132, In: A. F. Dyer, ed. *The Experimental Biology of Ferns*. London: Academic Press.
- WU, S. H. 1989. Materials of Chinese Aspleniaceae II. *Guihaia* 9:289–292.
- WU, S. H. 1999. *Flora Reipublicae Popularis Sinicae* 4(2). Science Press, Beijing.
- YATABE, Y., S. MASUYAMA, D. DARNAEDI and N. MURAKAMI. 2001. Molecular systematics of the *Asplenium nidus* complex from Mt. Halimun National Parks, Indonesia: evidence for reproductive isolation among three sympatric *rbcL* sequence types. *Amer. J. Bot.* 88:1517–1522.